ELSEVIER

Biochemical Pharmacology 64 (2002) 285–288

Biochemical Pharmacology

Short communication

Hyperekplexia mutation of glycine receptors: decreased gating efficacy with altered binding thermodynamics

Gábor Maksay[a,*], Tímea Bíró[a], Bodo Laube[b]

[a]*Department of Molecular Pharmacology, Chemical Research Centre, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary*
[b]*Department of Neurochemistry, Max-Planck-Institute for Brain Research, 60528 Frankfurt/Main, Germany*

Received 6 March 2002; accepted 8 May 2002

Abstract

[^{3}H]Strychnine binding was studied to recombinant human α_1 and the hyperekplexia mutant α_1R271L glycine receptors (GlyRs) transiently expressed in human embryonic kidney (HEK)-293 cell cultures at 0, 18 and 37°. The α_1R271L mutation did not affect the linear van't Hoff plots of the exothermic binding of the antagonist [^{3}H]strychnine while it turned taurine into an antagonist with exothermic binding. The inhibition constants of the agonist glycine showed opposite temperature dependence on α_1 GlyRs, corresponding to endothermic binding driven by large entropic increases. The temperature dependence of displacement by the partial agonists taurine on α_1 GlyRs and glycine on α_1R271L GlyRs was biphasic reflecting negative heat capacity changes, dehydration changes and/or a complex binding mechanism. The thermodynamic discrimination of efficacy is valid for native rat spinal and recombinant human GlyRs. The α_1R271L mutation impairs the transduction mechanism and distorts gating of GlyRs. Thereby it reduces the potency and efficacy of agonists and affects their thermodynamic parameters of binding. The hyperekplexia mutation offers a model system to demonstrate the correlation among pathophysiology, gating efficacy and binding thermodynamics of GlyRs. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Recombinant glycine receptors; Hyperekplexia mutation; Thermodynamics of binding; Strychnine; Partial agonists; Taurine

1. Introduction

Glycine is the major inhibitory neurotransmitter in mammalian spinal cord. Glycine receptors (GlyRs) belong to the superfamily of ionotropic neurotransmitter receptors containing nicotinic acetylcholine-, $GABA_A$- and 5-HT_3-type serotonin receptors [1]. Five subunits of these receptors each with four transmembrane regions (TM1-4) encircle an ion channel. Various subunits called $\alpha_{1\text{-}4}$ and β have been cloned [1]. Adult GlyRs are predominantly heterooligomers of α_1 and β subunits [2]. The N-terminal extracellular region of α_1 subunits contains adjacent but overlapping binding sites for the agonist glycine and the antagonist strychnine [3]. Point mutations of α_1 subunits in and flanking TM2 are associated with inherited hyperekplexia or startle disease [4]. Hyperekplexia mutations α_1R271L/Q at the extracellular end of TM2 impair the gating of GlyRs and turn the agonists β-alanine and taurine into antagonists [5–7].

The temperature dependent displacement of [^{3}H]strychnine binding to GlyRs revealed distinctive differences in the thermodynamic driving forces for the binding of agonists vs. antagonists [8]. Thermodynamic discrimination of the gating efficacy has been found for all members of this ionotropic receptor superfamily such as $GABA_A$ [9], 5-HT_3 serotonin [10,11] and nicotinic acetylcholine receptors [12]. This has been attributed to the similarities of the gating mechanisms of these structurally related receptors [13,14]. The chaotropic anion thiocyanate elicits different entropy increases to drive the binding of antagonists, bicuculline vs. gabazine (SR 95531) to $GABA_A$ receptors [15]. The predominant role of the entropic term is also supported by the *temperature-nearly-independent* binding of recombinant $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors for which displacing potencies correlated with efficacy [16].

We demonstrate here that the hyperekplexia mutation R271L of recombinant α_1 GlyRs does not affect the thermodynamic driving forces of [^{3}H]strychnine binding. In contrast, this mutation alters the temperature dependence of the displacing potency of the agonists glycine and taurine, which can be attributed to the impairment of gating efficacy. Consequently, this point mutation offers a model

* Corresponding author. Tel.: +361-325-7900x282; fax: +361-325-7554.
E-mail address: maksay@chemres.hu (G. Maksay).

Abbreviations: GABA, γ-aminobutyric acid; GlyR, glycine receptor; 5-HT, 5-hydroxytryptamine; K_i, inhibition constant; TM, transmembrane.

PII: S0006-2952(02)01111-5

system to demonstrate the correlation among pathophysiology, gating efficacy and binding thermodynamics of GlyRs.

2. Materials and methods

2.1. Receptor preparation

Human embryonic kidney cells (HEK-293 cells, ATCC CRL 1573) were transfected with the human GlyR α_1 subunit cDNA and the mutant α_1R271L inserted into the mammalian expression vectors pCIS2 as described [7,17]. Cells were harvested after 48 hr of expression, suspended in 50 mM Tris-HCl buffer (pH 7.4), homogenized with Ultra-Turrax for 10 sec and centrifuged at 40,000 *g* for 15 min. The pellets were washed by a similar centrifugation and frozen.

2.2. Binding studies

Membrane suspensions in 50 mM Tris citrate buffer containing 100 mM KSCN were centrifuged at 40,000 *g* for 15 min. Membranes were suspended in 50 mM Tris citrate–100 mM KSCN at 0, 18 and 37° (pH adjusted to 7.4 at each temperature) and incubated with [^{3}H]strychnine for 15–45 min (referring to 37 and 0°, respectively). For saturation analysis the range of [^{3}H]strychnine concentrations was 0.2–30 nM at 0°, 1–80 nM at 18° and 1–150 nM at 37°. Triplicate 0.2 mL samples were diluted into 3 mL ice-cold Tris buffer, filtered immediately on Whatman GF/C filters under vacuum and rinsed with 3 × 3 mL Tris buffer. Nonspecific binding was determined in the presence of 10 μM strychnine. In displacement studies different concentrations of glycine or taurine were incubated with [^{3}H]strychnine (2 nM at 0°, 7 nM at 18° and 20 nM at 37°). Radioactivity of the filters was measured in Hisafe cocktail using scintillation spectrometry. Protein content was determined using the Bio-Rad reaction with bovine serum albumin as standard.

2.3. Data analysis

Nonlinear regression of the saturation of specific binding *via* GraphPad Prism 2 resulted in dissociation constants (K_d) and the number of binding sites (B_{max}) of [^{3}H]strychnine. Inhibition constants of the displacing agents were determined according to Eq. (1):

$$K_i = \frac{IC_{50}}{1 + c/K_d} \quad (1)$$

Linear van't Hoff plots ($-\ln K_i$ or $-\ln K_d$ vs. $1/T$) result in slope values of $\Delta H^\circ/R$. Gibbs free energy changes (ΔG°) and entropy changes (ΔS°) were calculated at 18° according to Eq. (2):

$$\Delta G^\circ = RT \ln K_i = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

When the van't Hoff plots were not linear, a semiquantitative determination of the thermodynamic parameters was performed *via* the polynomial function of T according to Eqs. (3)–(5) [18]:

$$\Delta G^\circ = A + BT - CT^2 \quad (3)$$

$$\Delta H^\circ = A - CT^2 \quad (4)$$

$$\Delta S^\circ = -B - 2CT \quad (5)$$

3. Results and discussion

Saturation analysis of [^{3}H]strychnine binding resulted in B_{max} values (in pmol/mg protein) of 7.3 ± 1.0, 5.9 ± 0.9 and 6.6 ± 1.9 for α_1 GlyRs, while those of α_1R271L GlyRs were 19.4 ± 1.4, 18.2 ± 1.4 and 18.9 ± 0.9 at 0, 18 and 37°, respectively (mean ± SEM of three experiments). K_d values increased with increasing temperature but did not differ for α_1 and α_1R271L GlyRs (Fig. 1A). The van't Hoff plots of the K_d values were linear (Fig. 1A). The slopes of the lines result in $\Delta H^\circ = -45.1 \pm 7.0$ kJ/mol and $\Delta H^\circ = -53.3 \pm 2.6$ kJ/mol for α_1 and α_1R271L GlyRs, respectively. These values are not significantly different and correspond to exothermic binding interactions for the antagonist strychnine. Minor changes in entropy were $\Delta S^\circ = -4 \pm 24$ J/mol K and $\Delta S^\circ = -32 \pm 9$ J/mol K for α_1 and α_1R271L receptors, respectively. Thus strychnine binding is driven by decreases in enthalpy and it is not affected significantly by the hyperekplexia mutation. These thermodynamic parameters are similar to those of [^{3}H]strychnine binding to native GlyRs in rat spinal cord [8].

The temperature dependence of the displacing potencies of glycine and taurine was also examined. Fig. 1B and C shows that the hyperekplexia mutation strongly increased the K_i values of these agonists. Interestingly, the van't Hoff plots (Fig. 1B and C) display similar deflections from linearity only for the agonists of recombinant human GlyRs, while van't Hoff plots have been linear for the majority of receptor binding data [12] including rat spinal GlyRs [8]. On the other hand, the van't Hoff plot of taurine was linear for α_1R271L GlyRs (Fig. 1B) for which taurine is an antagonist. The exothermic binding of taurine to α_1R271L GlyRs is associated with $\Delta H^\circ = -13.9 \pm 4.2$ kJ/mol and $\Delta S^\circ = 17 \pm 15$ J/mol K. In contrast, the K_i values of glycine for α_1 GlyRs decreased with increasing temperature (Fig. 1C). That is, the endothermic binding of this agonist is driven by increases in entropy. The K_i values of the partial agonists taurine for α_1 GlyRs and glycine for α_1R271L GlyRs showed biphasic temperature dependence with highest affinities around intermediate temperatures (Fig. 1B and C).

Fig. 2 displays the enthalpic and entropic components (ΔH° and $-T\Delta S^\circ$, respectively) of the driving forces of binding to GlyRs. Where van't Hoff plots were nonlinear the polynomial function of T [18] enabled a semiquanti-

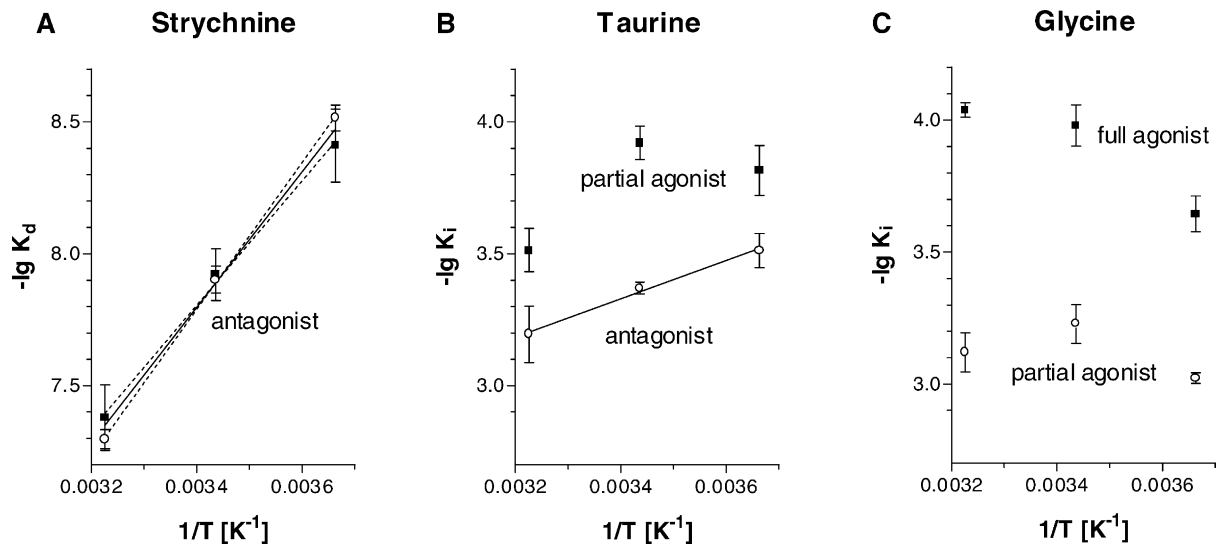

Fig. 1. The van't Hoff plots of [^{3}H]strychnine binding (A: K_d) and displacement (K_i) by taurine (B) and glycine (C) for recombinant human α_1 (■) and α_1R271L (○) GlyRs transiently expressed in HEK-293 cells. Data are mean ± SEM of three–seven experiments. (A) The fitted line and its standard error (dashed line) represent linear regression to the receptors in common with $\Delta H^\circ = -49.2 \pm 3.7$ kJ/mol and $\Delta S^\circ = -18 \pm 13$ J/mol K.

tative determination of the thermodynamic parameters at $T = 291$ K. The thermodynamic parameters of binding to rat spinal GlyRs [8] were also plotted in Fig. 2 for comparison. Our data on recombinant human GlyRs support the thermodynamic discrimination of efficacy. Binding of antagonists is driven by enthalpic decreases accompanied by slight changes in entropy, while binding of agonists is driven by large increases in entropy compensating the disadvantageous increases in enthalpy. The partial agonists taurine and β-alanine are situated in the middle with nearly isothermic binding and intermediate increases in entropy.

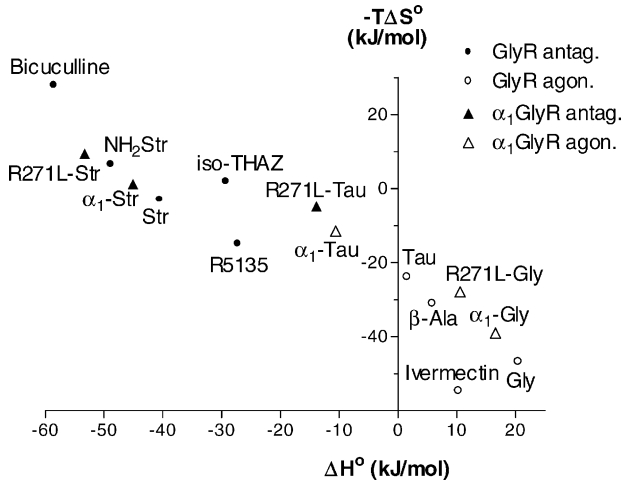

Fig. 2. The enthalpic (ΔH°) and entropic ($-T\Delta S^\circ$) components of the driving forces of binding to GlyRs at 291 K. Agonists and antagonists are indicated by open and closed symbols, respectively. Recombinant human α_1 and α_1R271L GlyRs are represented by triangles and native GlyRs of rat spinal cord [8] by circles. Abbreviations: strychnine (Str), taurine (Tau), alanine (Ala), glycine (Gly), 5,6,7,8-tetrahydro-4H-isoxazolo[5,4-c]azepin-3-ol (iso-THAZ).

There are various explanations for the existence or lack of correlation between the thermodynamic parameters of receptor binding and efficacy [12,19–21]. The hyperekplexia mutation helps to elucidate this correlation for GlyRs. The point mutation α_1R271L does not alter the loops contributing to the binding sites of strychnine and glycine [22] but it impairs the channel transduction mechanism [5,7]. Since agonist binding and channel gating are mutually coupled [23], the impairment of the channel transduction mechanism affects not only the gating efficacy but also the thermodynamic parameters of agonist binding. Since the α_1R271L mutation does not significantly affect the affinity and thermodynamic parameters of [^{3}H]strychnine binding, the altered thermodynamic parameters of agonist displacement should be attributed to efficacy-related changes of the receptor-ionophore.

Nonlinear van't Hoff plots were restricted to the agonists of recombinant GlyRs. This nonlinearity might be attributed to negative heat capacity changes, hydrophobic interactions and dehydration upon binding close to the surface of receptors [12,24–26]. Alternatively, the binding of agonist A to receptor R is made up of more than one step as suggested for insulin and 5-HT$_3$ receptors [10,24]:

$$A + R \leftrightarrow AR \leftrightarrow AR^* \quad (6)$$

Either way, it is reasonable to invoke agonist-elicited conformational changes of the receptor (R^*), i.e. the opening and/or desensitisation of the ionophore. This is associated with large variations in water-accessible receptor surface. The α_1R271L GlyR mutation impedes these conformational changes. The thermodynamic discrimination of efficacies for the ionotropic neurotransmitter receptor superfamily indicates a major contribution of functionally relevant transitions of ionophore states.

Acknowledgments

This work was supported by grants OTKA T 29723 and NKFP 1/047/2001. We thank Professor Heinrich Betz for continual support and stimulating discussions.

References

[1] Betz H. Ligand-gated ion channels in the brain: the amino acid receptor superfamily. Neuron 1990;5:383–92.
[2] Langosch D, Thomas L, Betz H. Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer. Proc Natl Acad Sci USA 1988;85:7394–8.
[3] Vandenberg RJ, Handford CA, Schofield PR. Distinct agonist- and antagonist-binding sites on the glycine receptor. Neuron 1992;9: 491–6.
[4] Shiang R, Ryan SG, Zhu YZ, Hahn AF, O'Connell P, Wasmuth JJ. Mutations in the α_1 subunit of the inhibitory glycine receptor cause the dominant neurologic disorder, hyperekplexia. Nat Genet 1993;5: 351–8.
[5] Rajendra S, Lynch JW, Pierce KD, French CR, Barry PH, Schofield PR. Mutation of an arginine residue in the human glycine receptor transforms β-alanine and taurine from agonists into competitive antagonists. Neuron 1995;14:169–75.
[6] Laube B, Langosch D, Betz H, Schmieden V. Hyperekplexia mutations of the glycine receptor unmask the inhibitory subsite for β-amino acids. Neuroreport 1995;6:897–900.
[7] Langosch D, Laube B, Rundström N, Schmieden V, Bormann J, Betz H. Decreased agonist affinity and chloride conductance of mutant glycine receptors associated with human hereditary hyperekplexia. EMBO J 1994;13:4223–8.
[8] Ruiz-Gómez A, García-Calvo M, Vázquez J, Marvizón JCG, Valdivieso F, Mayor F. Thermodynamics of agonist and antagonist interaction with the strychnine-sensitive glycine receptor. J Neurochem 1989;52:1775–80.
[9] Maksay G. Thermodynamics of γ-aminobutyric acid A-type receptor binding differentiate agonists from antagonists. Mol Pharmacol 1994;46:386–90.
[10] Maksay G. Distinct thermodynamic parameters of 5-HT_3 agonists and antagonists to displace [^{3}H]granisetron binding. J Neurochem 1996;67: 407–12.
[11] Borea PA, Dalpiaz A, Gessi S, Gilli G. Thermodynamics of 5-HT_3 receptor binding discriminates agonistic from antagonistic behavior. Eur J Pharmacol 1996;298:329–34.
[12] Borea PA, Varani K, Gessi S, Gilli P, Gilli G. Binding thermodynamics at the human neuronal nicotinic receptor. Biochem Pharmacol 1998;55:1189–97.
[13] Maksay G. Commentary. From kinetics and thermodynamics of $GABA_A$ receptor binding to ionophore function. Neurochem Int 1996;29:361–70.
[14] Maksay G. Comparison of ionotropic $GABA_A$, glycine and 5-HT_3-type serotonin receptor interactions. In: Raffa RB, editor. The thermodynamics of the drug-receptor interaction. Chichester: Wiley, 2001. pp. 359–76.
[15] Maksay G. Differential effects of thiocyanate on the binding thermodynamics of bicuculline methiodide versus SR 95531 (gabazine) to the γ-aminobutyric acid receptor-ionophore complex. Biochem Pharmacol 1998;56:729–31.
[16] Maksay G, McKernan R. Entropy as the predominant driving force of binding to human recombinant $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors. Eur J Pharmacol 2001;411:55–60.
[17] Laube B, Betz H. Purification and heterologous expression of inhibitory glycine receptors. Methods Enzymol 1998;294:260–73.
[18] Edelhoch H, Osborne JC. The thermodynamic basis of the stability of proteins, nucleic acids, and membranes. Adv Protein Chem 1976;30: 183–250.
[19] Weiland GA, Minneman KP, Molinoff PB. Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. Nature 1979;281:114–7.
[20] Franklin TJ. Binding energy and the activation of hormone receptors. Biochem Pharmacol 1980;29:853–6.
[21] Testa B, Jenner P, Kilpatrick GJ, El Tayar N, Van De Waterbeemd H, Marsden CD. Do thermodynamic studies provide information on both the binding to and the activation of dopaminic and other receptors? Biochem Pharmacol 1987;36:4041–6.
[22] Rajendra S, Vandenberg RJ, Pierce KD, Cunningham AM, French PW, Barry PH, Schofield PR. The unique extracellular disulfide loop of the glycine receptor is a principal ligand binding element. EMBO J 1995;14:2987–98.
[23] Colquhoun D. Binding, gating, affinity and efficacy: the interpretation of structure–activity relationships for agonists and of the effects of mutating receptors. Br J Pharmacol 1998;125:923–47.
[24] Waelbroeck M, Van Obberghen E, De Meyts P. Thermodynamics of the interaction of insulin with its receptor. J Biol Chem 1979;254: 7736–40.
[25] Ross PD, Subramanian S. Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 1981;20: 3096–102.
[26] Miklavc A. Temperature-nearly-independent binding constant in several biochemical systems. Biochem Pharmacol 1996;51:723–9.